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REPRODUCTION STUDY - RABBITS

MONOSODIUM GLUTAMATE

FINAL REPORT

Submitted to

International Minerals and Chemical Corp.
Skokie, Illinois

November 3, 1966

HAZLETON LABORATORIES, INCORPORATED

FALLS CHURCH, VIRGINIA

Sponsor: International Minerals & Chemical Corporation Date: November 3, 1966

Material: Monosodium Glutamate Lot No:

Subject: FINAL REPORT
Reproduction Study - Rabbits
Project No. 466-103

INTRODUCTION

The purpose of this study was to characterize and evaluate, in adult albino rabbits, the toxicity of monosodium glutamate following short-term (six to seven weeks) dietary feeding; the effect upon reproduction after two to three weeks ingestion of monosodium glutamate; and the teratogenic potential of monosodium glutamate.

MATERIAL

The samples of monosodium glutamate which were used in this study were received from International Minerals & Chemical Corporation on January 19, 1966, May 2, 1966, and May 12, 1966. They were submitted by the sponsor as routine commercial production samples, labeled ACCENT brand monosodium glutamate, fine crystalline grade. The materials were white powders with no discernible odor. For the purpose of this study the compound was considered to be free of impurities and was used as received.

METHODS

One hundred and eighteen healthy proven breeding does and 64 healthy proven breeding bucks, all of the New Zealand white variety, were used in the study. Each animal was known to have produced or sired at least one litter prior to the test. Initial body weights ranged from 2.6 to 4.4 kg. for the females and from 3.0 to 4.7 kg. for the males. The animals were randomly divided into the following five groups:

Group No.	No. of Animals		Dietary Level	
	females	males	%	mg/kg
1 (Negative Control)	24	16	0	0
2 (Positive Control)	22	0	-	100*
3 (Low Level)	24	16	0.1	-
4 (Middle Level)	24	16	0.825	-
5 (High Level)	24	16	8.25	-

The animals were acclimated to laboratory conditions and to a basal diet of ground Purina Rabbit Chow for one to two weeks prior to the start of the study. After acclimation, both the negative and positive control animals were maintained on the basal ground diet. For the test animals, MSG was incorporated into the basal diet on a weight/weight basis and thoroughly mixed in a twin-shell blender to provide the desired dietary levels. Fresh diets were prepared twice weekly. Apart from Group No. 2, the

* From Day 8 to Day 17 of the gestation period.

appropriate diet and water were available to all male and female animals until sacrifice.

After a two to three week feeding period, each doe in Groups No. 1, No. 3, No. 4, and No. 5 was mated with a breeding buck from the corresponding group; mating was repeated on the following day to ensure fertilization. Each doe in Group No. 2 was also mated in the same manner; however, bucks from the supplier's breeding colony were used and then returned. An equal number of does (eight) from each group were bred at three different times, within a period of one week; eight of the bucks in each group were used at both the first and the third breeding intervals. From the eighth through the 16th day of the gestation period (calculated from the second mating) each doe in Group No. 2 received Thalidomide at a dose of 100 mg/kg of body weight per day. The material was mixed in the ground food during this time; on the 17th day, these animals were returned to the untreated basal diet.

From the eighth through the 16th day of the gestation period, individual body weights and food consumption were recorded daily for the animals in Group No. 2. Otherwise, individual body weights were recorded weekly and food consumption was measured and recorded three times weekly. The animals were observed for gross toxic signs and general appearance and behavior daily throughout the study.

On the 29th or 30th day of gestation, all does were sacrificed by intravenous air embolism and the young immediately delivered by

Caesarean section. All litters were very carefully examined for stillbirths and possible malformations. Individual weights (in grams) and length (in centimeters) were recorded for all fetuses. The uteri of all does were carefully examined for sites of fetal attachment and resorption. Each fetus was sacrificed by chloroform, examined grossly, and then placed in 10% formalin. At this time each male animal was also sacrificed by intravenous air embolism and necropsied. At necropsy, gonads from representative male animals in each group, as well as any grossly abnormal organs from either sex were placed in 10% formalin. These tissues are being stored at Hazleton Laboratories, Inc., for possible future reference.

After fixation, approximately one-third of the fetuses from each litter were inspected for external and visceral abnormalities and prepared for skeletal clearing and staining as follows: each fetus was skinned, eviscerated, and then totally immersed in a 2.0% solution of KOH for maceration; after a period of three to four weeks, depending on fetal size, the fetuses were transferred to a solution of Alizarin Red S for staining; and after complete staining, the fetuses were cleared in glycerin and held in this solution for observation of skeletal malformations and abnormalities. Bone structure development evaluations included the cranial and facial aspects of the skull, pectoral girdle, anterior limbs, sternum, and posterior limbs. These fetuses, as well as the ones which were not cleared or stained, are being held at Hazleton Laboratories, Inc.

RESULTS

The mean weekly body weights, weight ranges, and food consumption, as well as the amount of compound consumed on average mg/kg/day basis are presented in Table No. 1. The positive control females (Group No. 2) are not included in this table since they received compound only from the eighth through the 16th gestation day.

In general, body weight gains and food consumption were comparable among all groups. Slight body weight losses and decreased food consumption were noted during the transient periods when signs of respiratory and/or intestinal infection were noted in a few of the animals. A marked body weight loss (note low 1575 gram range in Table No. 1 for 0.1% females) in the animal that died was observed. Except for the interval (eighth through the 16th day) when Thalidomide was administered and food was restricted (100 grams/animal/day) to assure complete compound consumption, the body weight gains and food consumption for the positive control animals were comparable with the negative control animals.

General Appearance and Behavior

At the 0.1% level, one animal of each sex (female Rabbit No. 66-809 and male Rabbit No. 66-900) died during the third or sixth week of the study; gross signs prior to death (depression, labored respiration, bloating, mucoid diarrhea, and body weight loss) and necropsy findings indicated

mucoid enteritis, a coincidental disease not related to oral ingestion of the compound. Gross signs indicating mucoid enteritis were also noted in one high level male during the last week or two of the study.

One positive control animal aborted on the 19th gestation day and was therefore sacrificed at that time. Doe No. 66-839 (0.825% monosodium glutamate level) was sacrificed on the 25th gestation day due to a moribund condition, and one negative control animal was sacrificed on the 27th gestation day after one fetus was found in the cage.

Apart from these findings, and except for transient signs of wheezing and/or a nasal discharge and soft feces observed in both control and test animals, all animals seemed normal in appearance and behavior throughout the study.

Gross Pathology - Parents

One negative control male animal showed apparent atrophy of one testis. The following necropsy findings were noted among the female negative control animals: friable, mottled, and granular liver (one animal); parasitic areas on the liver, parasitic cysts on the mesentery, and fluid in the peritoneal cavity (one animal); and no grossly visible right uterine horn (one animal).

At the 0.1% level, the male animal that died showed marked autolytic changes, congested lungs, and ruptured stomach; the female animal that died showed congested lungs, parasitic areas on the liver, and hardened fecal material in the cecum. Watery cysts were noted in the uterine wall of one female animal at necropsy following sacrifice.

At the 0.825% level the entire right lung of the animal sacrificed on the 29th day was consolidated and abscessed. One 8.25% male animal showed pitted, congested, and tough kidneys, gelatinous intestinal contents, and slight intestinal inflammation. One nonpregnant high level female showed uterine inflammation, and one pregnant high level female showed watery cysts throughout the renal cortex. No other gross necropsy findings were noted in any of the control or test animals.

Breeding Data

The breeding data, which include days of gestation, method of delivery (Caesarean or natural), number of implantation and resorption sites, number of live young and stillbirths, and mean litter weights (in grams) and lengths (in centimeters) for control and test animals are presented in Table No. 2.

Negative Control:

Twenty-one of the 24 does in this group became pregnant. Apart from the Caesarean section performed on the 27th day after one fetus was expelled, Caesarean deliveries were performed on the 29th or 30th day. The uteri of the three nonpregnant animals did not show any evidence of fetal implantation or resorption. Total implantation sites in the pregnant does of this group averaged 8.0 and ranged from one to 14. Of the 168 fertilized ova implanted, 19 were resorbed, 132 were born alive, 17 (including the one expelled) were dead, and five showed gross abnormalities. One of the live

pups from Doe No. 66-771 showed collapsed lungs and an incomplete formation of the diaphragm with adhesions between liver tissue and thoracic tissue. Two of the live pups from Doe No. 66-760 showed milky abdominal fluid and intestinal adhesions. The one expelled fetus from Doe No. 66-754 showed incomplete skin formation over the anterior part of the body, including the head, no grossly visible bone formation over the cranial area, a small, twisted left front paw, and a turning down of the right forepaw. One dead pup from Doe No. 66-761 showed a mottled liver, pale-appearing intestines, and a small amount of fluid in the thoracic cavity. The dead fetuses varied in size, depending on the stage of development prior to death, and only three were comparable to the live fetuses in size. No other gross abnormalities were observed among the other fetuses.

Positive Control:

Eighteen of the 22 does in this group became pregnant; however, Doe No. 66-787 aborted nine small embryos on the 19th gestation day, and the uteri of Doe No. 66-786 showed, when sacrificed on the 29th gestation day, six apparent implantation sites which could not be accounted for. Possibly this doe aborted sometime during the gestation period and devoured the young. The uteri of the four nonpregnant animals did not show any evidence of fetal implantation or resorption.

Total implantation sites, including the six unaccounted for sites noted above, in the pregnant does of this group averaged 8.0 and ranged from

three to 12. Of the 148 fertilized ova implanted, eight were resorbed; 25, including the nine aborted on the 19th day, were born dead; 109 were born alive; and six were unaccounted for. Eight of the young, seven live ones and one dead one, showed gross abnormalities.

One of the dead fetuses from Doe No. 66-782 showed apparent umbilical strangulation. One of the live young from Doe No. 66-778 showed a watery-appearing cyst and incomplete skin formation over the sacrum and displayed very little use of the hind limbs. Six of the remaining live young, two from Doe No. 66-785 and one each from Does No. 66-791, No. 66-794, No. 66-795, and No. 66-796, showed gross abnormalities of the left or right forepaw; a bent or club-like appearance was observed and one pup walked on the radial-carpal joint. The one pup from Doe No. 66-794 also showed a diaphragmatic hernia resulting in a portion of the intestines present in the thoracic cavity, collapsed lungs, and the absence of a right kidney. The dead fetuses varied in size, depending on the stage of development prior to death, and none were comparable to the live fetuses in size. No other gross abnormalities were observed among the other fetuses.

0.1% Level:

Nineteen of the 24 does in this group became pregnant. The uteri of the five nonpregnant does, including the one that died, did not show any evidence of fetal implantation or resorption. Total implantation sites in the pregnant does of this group averaged 9.5 and ranged from six to 13. Of the 181 fertilized ova implanted, 12 were resorbed, nine were born dead, and 160

were born alive. Two of the does (Does No. 66-816 and No. 66-823) delivered live pups prior to scheduled Caesarean sections. One of the pups from Doe No. 66-816, natural delivery, showed what appeared to be subcutaneous hemorrhage in the cranial area. No other gross external or internal abnormalities were observed in any of the live or dead fetuses. The dead fetuses varied in size, depending on the stage of development prior to death, and none were comparable to the live fetuses in size.

0.825% MSG:

Twenty of the 24 does in this group became pregnant. For Doe No. 66-839 pregnancy was terminated on the 25th gestation day due to a moribund condition; Doe No. 66-832 delivered naturally before scheduled Caesarean section. The uteri of the four nonpregnant animals did not show any evidence of fetal implantation or resorption.

Total implantation sites in the pregnant does of this group averaged 8.6 and ranged from three to 11. Of the 173 fertilized ova implanted, 14 were resorbed, eight were born dead, and 151 were born alive. No gross internal or external abnormalities were observed in any of the live or dead fetuses. The dead fetuses varied in size, depending on the stage of development prior to death, and only two of them were comparable to the live fetuses in size; the litter delivered on the 25th gestation day was slightly smaller in size than those obtained on the 29th and 30th days.

8.25% MSG:

Eighteen of the 24 does in this group became pregnant. The uteri of the six nonpregnant animals did not show any evidence of fetal implantation or resorption. Total implantation sites in the pregnant does of this group averaged 9.3 and ranged from 7 to 11. Of the 168 fertilized ova implanted, eight were resorbed, 16 were born dead, and 144 were born alive. The dead fetuses varied in size, depending on the stage of development prior to death, and only one of these was comparable in size to the live fetuses of the same litter. One of the live young from Doe No. 66-852 showed a malformed forepaw and walked on the radial-carpal joint. No gross internal or external abnormalities were observed in any of the other fetuses.

Skeletal Staining

Individual bone changes for all control and test pups which were cleared are presented in Table No. 3.

Clearing and staining was performed on 47 negative control pups, 42 positive control pups, 53 of the 0.1% test pups, 48 of the 0.825% test pups, and 49 of the 8.25% test pups.

After staining, the bone structure was evaluated for any gross or comparative difference in size, location, atypical formation, and incomplete ossification, and for the abnormal absence or presence of bone structures.

In the negative control group, skeletal abnormalities noted in three of the pups included marked fusion of ribs on the left side in the

lumbar region (one live pup), and slight or moderate spinal curvature in the thoracic region (two dead pups). Other apparently spontaneously occurring findings found in these control animals as well as in control animals from previous studies included missing or small fifth sternebrae, small sixth sternebrae, and small 13th rib.

In the positive control group, no skeletal abnormalities, apart from those frequently found in negative control animals, were found in any of the pups cleared.

At the 0.1% test level, bone changes were comparable to those found in the negative control animals; however, ribs 8 - 9 of one pup showed a swollen area in the central portion. At the 0.825% test level, no skeletal abnormalities were found in any of the pups cleared, bone changes were comparable to those occurring spontaneously in control pups. At the 8.25% test level, three live pups from Doe No. 66-855 showed retarded closure of the cranial sutures. Since these latter changes were found in a single litter and not in others at this dosage, it is not considered to be compound-induced but probably due to genetic alteration. Retarded suture closure is not a frequent spontaneous anomaly in this strain of rabbits. No other skeletal abnormalities other than those also occurring in the control animals, were noted in any of the pups cleared.

SUMMARY

Oral ingestion of monosodium glutamate for six to seven weeks at dietary feeding levels of 0.1, 0.825, and 8.25% did not produce, in adult male and female albino rabbits, any toxic effects which could be attributed to compound. Transient signs of respiratory and intestinal infection were noted in both control and test animals. At the 0.1% level, one animal of

each sex died during the study; gross signs prior to death and necropsy findings indicated intestinal mucoid enteritis. One 0.825% doe sacrificed on the 29th gestation day in a moribund condition showed lung consolidation and abscessation. At termination one high level male showed gross signs of mucoid enteritis and gelatinous intestinal contents at necropsy. Body weight gains and food consumption were comparable in all groups. The test males and females were fed monosodium glutamate two to three weeks prior to mating, and were mated to animals within the same group. The positive control animals received 100 mg. of Thalidomide per kg. of body weight from the eighth through the 16th gestation day only and were maintained on ground Purina Rabbit Chow during the remainder of the study, while the negative control animals received the ground basal diet during the entire study. Conception occurred in 22/24 negative control does, 18/22 positive control does, 19/24 0.1% test does, 20/24 0.825% test does, and 18/24 8.25% test does.

The gross internal and external abnormal findings and number of resorption sites per total number of implantation sites are summarized below.

GROUP	GROSS INTERNAL ABNORMALITIES		GROSS EXTERNAL ABNORMALITIES		RESORP-TIONS
	Live Fetuses	Dead Fetuses	Live Fetuses	Dead Fetuses	
Negative Control	3/132	1/17	0/132	1/17	19/168
Positive Control	1/109	0/16	7/109	1/16	8/148
0.1% MSG	0/160	0/9	1/160	0/9	12/181
0.825% MSG	0/151	0/8	0/151	0/8	14/173
8.25% MSG	0/144	0/16	1/144	0/16	8/168

The total number of dead positive control fetuses used in the above summary does not include the fetuses aborted on the 19th day since they were too small for gross examination, nor the six unaccounted for implantation sites in the one doe. The one abnormal live fetus in the 0.1% MSG group was delivered naturally and showed, what appeared to be, subcutaneous hemorrhages in the cranial area. The gross external abnormalities noted among the seven live positive control fetuses and the one live 8.25% MSG fetus were malformed forepaws. The gross internal abnormalities noted in the live negative control fetuses included diaphragmatic hernia and collapsed lungs; and milky abdominal fluid and intestinal adhesions. One live positive control fetus also showed a diaphragmatic hernia. In one dead negative control animal (aborted on the 27th day) the gross external abnormalities consisted of malformed forepaws, incomplete skin formation over the anterior part of the body, and no apparent bone formation in the cranial area. In the one dead positive control animal, the gross external abnormalities consisted of umbilical strangulation. The gross internal abnormalities in the one dead negative control fetus included a mottled liver, pale-appearing intestines, and a small amount of fluid in the thoracic cavity. The average litter weight of the live pups from each group ranged from 38 to 44 grams and the average length from 9.9 to 10.3 centimeters.

The type and incidence of skeletal abnormalities noted in all groups were consistent with the control experience of this laboratory for the rabbit strain used in this study.

PATHOLOGY REPORT

ADDENDUM TO FINAL REPORT DATED NOVEMBER 3, 1966
(REPRODUCTION STUDY - RABBITS)

PROJECT NO. 466-103

Submitted to

International Minerals & Chemical Corporation
Skokie, Illinois

HAZLETON LABORATORIES, INC.
A SUBSIDIARY OF TRW INC.
P.O. BOX 30, FALLS CHURCH, VIRGINIA 22046

July 18, 1969

HAZLETON LABORATORIES, INC.
TRW LIFE SCIENCES CENTER

Sponsor: International Minerals & Chemical Corporation Date: July 18, 1969

Material: Monosodium Glutamate

Subject: ADDENDUM TO FINAL REPORT
Reproduction Study - Rabbits
Project No. 466-103

SUMMARY

Microscopic examination was performed on hematoxylin- and eosin-stained sections of brain tissue from rabbit pups of Project No. 466-103, removed by Caesarean section on Gestation Day 29 or 30. Ten of the pups (five male and five female, Path No. 47-738 to Path No. 47-747) were removed from does which had been fed monosodium glutamate at a level of 8.25% for two to three weeks prior to mating and during the subsequent 29 to 30 days prior to Caesarean section. The 10 control pups (five males and five females) were obtained from does which were maintained on the basal laboratory diet. Transverse sections of the brain were examined at the level of the hypothalamic nuclei and pituitary gland. Artifactual distortion of the brain was occasionally noted in sections from pups of both treated and control does. Evidence of neuronal necrosis or other pathologic alteration was not apparent in the sections examined.

In conclusion, there was no discernible difference in the histologic appearance of brain sections from pups obtained from control does or those receiving monosodium glutamate at a level of 8.25% of the diet.

Submitted by Richard W. Voelker Jr.
RICHARD W. VOELKER, Jr., D.V.M., Ph.D.
Staff Pathologist

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CONCLUSION

Dietary feeding of Monosodium glutamate to adult male and female albino rabbits at levels of 0.1, 0.825, and 8.25% for two to three weeks prior to mating and during the subsequent 29 to 30 days produced no apparent effect in either sex. Body weight gains, food consumption, general appearance and behavior, survival, breeding performance, and all litter data (total number, number of live young, number of dead young, number of resorptions, gross internal and external abnormalities, and skeletal staining) were comparable among negative control and test animals. The incidence of grossly appearing malformed forepaws in the positive control (Thalidomide) animals was higher than in the other groups; however, skeletal staining did not confirm this finding and the effect was apparently not due to a bone abnormality.

Submitted by

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and average compound consumption on an mg/kg/day basis for male and female albino rabbits which served as negative controls or received Monosodium glutamate at the indicated dosage levels.

WEEK NO.	BODY WEIGHT IN GRAMS				MEAN FOOD CONSUMPTION (GRAMS)		COMPOUND CONSUMPTION (MG/KG/DAY)	
	MEAN males	RANGE males	MEAN females	RANGE females	males	females	males	females
				GROUP NO. 1 - NEGATIVE CONTROL				
0	3779	3026-4504	3386	2610-4358	-	-	-	-
1	3800	2970-4470	3406	2814-4496	771	783	-	-
2	3756	2951-4380	3374	2677-4370	699	697	-	-
3	3808	2951-4525	3492	2857-4496	871	892	-	-
4	3813	2971-4444	3542	2807-4624	851	888	-	-
5	3845	2969-4486	3676	2963-4927	818	952	-	-
6	3852	2918-4548	3736	3007-4798	764	803	-	-
7	3816	2900-4450	3699	3113-4218	797	936	-	-
				GROUP NO. 3 - 0.1% MONOSODIUM GLUTAMATE				
0	3697	3363-4200	3479	2677-4440	-	-	-	-
1	3698	3440-4252	3425	2526-4343	672	769	26.0	31.8
2	3664	3322-4218	3470	2022-4306	519	766	20.1	31.7
3	3736	3441-4310	3523	1575-4454	893	886	34.5	36.2
4	3764	3420-4341	3718	2985-4579	875	936	33.3	36.9
5	3791	3443-4501	3839	2991-4865	805	963	30.4	36.4
6	3814	3402-4460	3893	3023-4912	834	890	31.3	32.9
7	3946	3533-4470	3839	3102-4944	815	1109	30.6	41.0

WEEK NO.	BODY WEIGHT IN GRAMS				MEAN FOOD CONSUMPTION (GRAMS)		COMPOUND CONSUMPTION (MG/KG/DAY)	
	MEAN males	RANGE males	MEAN females	RANGE females	males	females	males	females
				GROUP NO. 4 - 0.825% MONOSODIUM GLUTAMATE				
0	3645	3014-4475	3129	2673-3927	-	-	-	-
1	3699	3100-4472	3204	2654-4123	677	840	217	313
2	3614	2977-4243	3223	2471-4076	512	767	165	281
3	3644	3131-4339	3320	2512-4125	830	985	270	355
4	3712	3158-4463	3475	2713-4262	808	1015	259	352
5	3730	3134-4500	3608	2760-4396	817	999	259	332
6	3742	3116-4462	3688	2854-4298	737	926	232	299
7	3480	3098-4134	3674	2784-4149	757	1151	247	368
				GROUP NO. 5 - 8.25% MONOSODIUM GLUTAMATE				
0	3713	2980-4671	3300	2674-4411	-	-	-	-
1	3748	2985-4643	3322	2752-4375	799	798	2520	2840
2	3696	3127-4466	3292	2699-4177	689	774	2180	2760
3	3809	3333-4694	3393	2907-4291	900	922	2830	3290
4	3813	3397-4584	3499	3095-4298	910	930	2810	3180
5	3841	3482-4515	3605	3201-4550	826	960	2540	3185
6	3877	3452-4580	3692	3236-4767	783	853	2390	2770
7	3823	3511-4551	3789	3426-4744	982	1060	3010	3340

or positive controls or received Monosodium Glutamate at the indicated dietary levels. See text for details.

DOE NUMBER	DELIVERY	GESTATION DAY	IMPLANTATION SITES	LIVE BIRTHS			STILLBIRTHS			
				NUMBER FETUSES	MEAN WEIGHT	MEAN LENGTH	NUMBER FETUSES	MEAN WEIGHT	MEAN LENGTH	RESORPTION SITES
GROUP NO. 1 - NEGATIVE CONTROL										
66-754	Caesarean	27	8	6	30	9.4	2*	-	-	0
66-755	Caesarean	29	12	11	46	11.3	1	-**	4.0	0
66-756	Caesarean	29	4	2	55	7.5	0	-	-	2
66-757	Caesarean	29	8	2	36	9.0	0	-	-	6
66-758	Caesarean	29	10	8	44	10.2	2	34	9.5	0
66-760	Caesarean	29	8	8	39	10.4	0	-	-	0
66-763	Caesarean	29	5	3	30	10.0	1	-**	4.0	1
66-764	Caesarean	29	8	5	47	10.5	2	14	7.0	1
66-765	Caesarean	29	14	10	32	9.5	3	18ϕ	5.7	1
66-766	Caesarean	29	8	7	46	11.3	1	-**	2.0	0
66-770	Caesarean	29	12	9	41	10.1	2	-**	3.5	1
66-771	Caesarean	29	3	2	45	10.0	0	-	-	1
66-772	Caesarean	29	8	8	39	9.9	0	-	-	0
66-773	Caesarean	29	12	12	40	9.7	0	-	-	0
66-761	Caesarean	30	6	4	55	10.9	1	46	11.0	1
66-767	Caesarean	30	7	5	43	10.3	1	-**	1.0	1
66-768	Caesarean	30	11	9	47	10.3	1	14	7.0	1
66-769	Caesarean	30	7	6	46	10.2	0	-	-	1
66-774	Caesarean	30	1	1	70	13.5	0	-	-	0
66-775	Caesarean	30	9	9	44	10.1	0	-	-	0
66-777	Caesarean	30	7	5	56	11.8	0	-	-	2
66-759	Not pregnant									
66-762	Not pregnant									
66-776	Not pregnant									

DOE NUMBER	DELIVERY	GESTATION DAY	IMPLANTATION SITES	LIVE BIRTHS NUMBER FETUSES	LIVE BIRTHS MEAN WEIGHT	LIVE BIRTHS MEAN LENGTH	STILLBIRTHS NUMBER FETUSES	STILLBIRTHS MEAN WEIGHT	STILLBIRTHS MEAN LENGTH	RESORPTION SITES
GROUP NO. 2 - POSITIVE CONTROL - 100 MG/KG/DAY THALIDOMIDE										
66-787	Early abortion	19	9ϕϕ							0
66-778	Caesarean	29	10	9	36	9.3	1	15	7.0	0
66-780	Caesarean	29	8	8	39	9.3	0	-	-	0
66-782	Caesarean	29	9	6	45	10.3	2	16ϕ	4.8	1
66-783	Caesarean	29	7	6	43	9.6	0	-	-	1
66-784	Caesarean	29	3	1	58	11.0	0	-	-	2
66-785	Caesarean	29	8	8	39	9.3	0	-	-	0
66-786	Caesarean	29	6ϕϕ	-	-	-	-	-	-	-
66-788	Caesarean	29	11	10	40	10.7	1	14	7.5	0
66-790	Caesarean	29	8	4	37	10.1	4	23ϕ	5.6	0
66-791	Caesarean	29	8	6	34	9.8	1	-**	5.0	1
66-792	Caesarean	29	12	9	41	10.1	2	5.3	5.8	1
66-793	Caesarean	29	10	9	37	9.9	1	-**	2.5	0
66-794	Caesarean	29	11	9	35	10.2	2	20	6.5	0
66-795	Caesarean	29	9	7	40	10.6	0	-	-	2
66-796	Caesarean	29	6	6	39	9.9	0	-	-	0
66-797	Caesarean	29	8	6	47	10.7	2	-**	5.3	0
66-799	Caesarean	29	5	5	50	11.5	0	-	-	0
66-779	Not pregnant									
66-781	Not pregnant									
66-789	Not pregnant									
66-798	Not pregnant									

DOE NUMBER	DELIVERY	GESTATION DAY	IMPLANTATION SITES	LIVE BIRTHS			STILLBIRTHS			
				NUMBER FETUSES	MEAN WEIGHT	MEAN LENGTH	NUMBER FETUSES	MEAN WEIGHT	MEAN LENGTH	RESORPTION SITES
GROUP NO. 3 - 0.1% MONOSODIUM GLUTAMATE										
66-802	Caesarean	29	9	7	48	11.4	0	-	-	2
66-803	Caesarean	29	7	7	56	12.0	0	-	-	0
66-805	Caesarean	29	6	5	37	9.5	0	-	-	1
66-806	Caesarean	29	9	9	38	10.1	0	-	-	0
66-808	Caesarean	29	13	10	31	8.5	2	20ϕ	5.3	1
66-810	Caesarean	29	9	5	36	9.6	0	-	-	4
66-811	Caesarean	29	10	8	37	10.4	2	4.8	5.3	0
66-812	Caesarean	29	10	7	39	9.5	3	-**	3.2	0
66-814	Caesarean	29	10	10	33	10.1	0	-	-	0
66-815	Caesarean	29	11	11	40	9.9	0	-	-	0
66-818	Caesarean	29	10	9	28	8.7	1	-**	2.0	0
66-820	Caesarean	29	10	9	35	9.3	0	-	-	1
66-807	Caesarean	30	12	12	35	9.5	0	-	-	0
66-813	Caesarean	30	10	9	39	9.9	1	11	6.5	0
66-817	Caesarean	30	9	9	41	9.7	0	-	-	0
66-821	Caesarean	30	8	7	45	11.3	0	-	-	1
66-822	Caesarean	30	8	7	29	8.8	0	-	-	1
66-823	Natural	29	11	11	39	9.6	0	-	-	0
66-816	Natural	30	9	8	39	9.6	0	-	-	1
66-809	Not pregnant (Died)									
66-804	Not pregnant									
66-819	Not pregnant									
66-824	Not pregnant									
66-825	Not pregnant									

DOE NUMBER	DELIVERY	GESTATION DAY	IMPLANTATION SITES	LIVE BIRTHS			STILLBIRTHS			
				NUMBER FETUSES	MEAN WEIGHT	MEAN LENGTH	NUMBER FETUSES	MEAN WEIGHT	MEAN LENGTH	RESORPTION SITES
GROUP NO. 4 - 0.825% MONOSODIUM GLUTAMATE										
66-839	Caesarean	25	10	8	18	7.8	2	20	7.5	0
66-826	Caesarean	29	10	7	33	10.3	0	-	-	3
66-827	Caesarean	29	8	8	44	10.7	0	-	-	0
66-828	Caesarean	29	9	6	42	10.4	0	-	-	3
66-829	Caesarean	29	11	10	37	9.7	0	-	-	1
66-830	Caesarean	29	11	9	45	10.2	0	-	-	2
66-835	Caesarean	29	7	6	34	10.1	0	-	-	1
66-836	Caesarean	29	5	5	38	10.3	0	-	-	0
66-837	Caesarean	29	7	5	42	9.9	2	-**	5.3	0
66-838	Caesarean	29	9	9	41	10.5	0	-	-	0
66-842	Caesarean	29	9	6	30	9.1	1	-**	1.0	2
66-843	Caesarean	29	11	11	35	9.5	0	-	-	0
66-831	Caesarean	30	8	7	47	10.4	0	-	-	1
66-833	Caesarean	30	9	8	42	10.1	1	10	6.0	0
66-834	Caesarean	30	3	2	63	11.3	1	-**	1.0	0
66-845	Caesarean	30	9	9	43	11.1	0	-	-	0
66-847	Caesarean	30	10	10	34	9.2	0	-	-	0
66-848	Caesarean	30	9	9	44	11.1	0	-	-	0
66-849	Caesarean	30	9	8	33	9.9	1	-**	3.0	0
66-832	Natural	30	9	8	47	10.0	0	-	-	1
66-840	Not pregnant									
66-841	Not pregnant									
66-844	Not pregnant									
66-846	Not pregnant									

groups serving as negative or positive controls (Thalidomide) or receiving Monosodium Glutamate; expressed as a percentage of number of fetuses showing variations/number of fetuses examined. Numbers in parentheses indicate total number of fetuses examined.

SKELETAL VARIATIONS	GROUP NO. 1 NEGATIVE CONTROL		GROUP NO. 2 POSITIVE CONTROL		GROUP NO. 3 0.1%		GROUP NO. 4 0.825%		GROUP NO. 5 0.25%	
	Alive (46) %	Dead (9) %	Alive (36) %	Dead (6) %	Alive (49) %	Dead (5) %	Alive (45) %	Dead (3) %	Alive (72) %	Dead (9) %
Sternebrae										
Missing: no. 1				17	2	40				
no. 2				17		20				
no. 3					2					
no. 4					2	20				
no. 5	9	22	3	50	6	20	20	33	20	67
no. 6	2			33	6	40	2	33	2	67
all (nos. 1 - 6)		44		33				33		
Small: no. 1								33		
no. 2		11	3		12	20	4	67	13	33
no. 3						40		33		33
no. 4						20		33		
no. 5	57	11	58	17	82	80	56	33	72	33
no. 6	4	22	3		4	20	7		11	
Additional: no. 7	2									
Fusion: nos. 1, 2, & 3	2									
nos. 4 & 5			3		2					
Atypical Structure: no. 5	2				2					
no. 6	4	11								
Ribs										
Thickened Areas: no. 7		11								
nos. 8 & 9					2					
all (nos. 1 - 12)	4									
Additional: no. 13	54	22	17		45	60	31	33	54	67
Small: no. 1			3			20		33		
no. 2	2									
Atypical Structure: no. 11			3							
no. 13										4
Fusion: all (nos. 1 - 12 on left)	2									

SKELETAL VARIATIONS	GROUP NO. 1 NEGATIVE CONTROL		GROUP NO. 2 POSITIVE CONTROL		GROUP NO. 3 0.1%		GROUP NO. 4 0.5%		GROUP NO. 5 2.0%	
	Alive (46) %	Dead (9) %	Alive (50) %	Dead (6) %	Alive (49) %	Dead (5) %	Alive (45) %	Dead (5) %	Alive (45) %	Dead (3) %
Ossification Incomplete										
Skull		33		50		60		67		33
Mandible		33								
Palate		33		17						
Teeth		11								
Cervical and Thoracic Vertebrae		33		17						
Lumbar Vertebrae		33								
Sacral Vertebrae		11		17		40		33		
Caudal Vertebrae				17		40		33		
Carpus		22		67		60		67		33
Pelvis		33								
Tarsus		22		67		60		67		33
Phalanges		33		33		20				
Cranial Sutures									7	
Ossified Material Not Visible										
Teeth		33		67		40		33		
Sacral Vertebrae		33		33						
Caudal Vertebrae		44		50		40				
Carpus		22		17						
Tarsus		22		17						
Sternebrae Covered with additional ossified material									2	
Cage Surrounded by wave-like material (not bone related)	20	11								
Axial Curvature Of thoracic region		22							2	
Inter Disc Atypical structure of one	2								2	

PATHOLOGY REPORT

ADDENDUM TO FINAL REPORT DATED NOVEMBER 3, 1966
(REPRODUCTION STUDY - RABBITS)

PROJECT NO. 466-103

Submitted to

International Minerals & Chemical Corporation
Skokie, Illinois

HAZLETON LABORATORIES, INC.
A SUBSIDIARY OF TRW INC.
P.O. BOX 30, FALLS CHURCH, VIRGINIA 22046

July 18, 1969

HAZLETON LABORATORIES, INC.
TRW LIFE SCIENCES CENTER

Sponsor: International Minerals & Chemical Corporation Date: July 18, 1969

Material: Monosodium Glutamate

Subject: ADDENDUM TO FINAL REPORT
Reproduction Study - Rabbits
Project No. 466-103

SUMMARY

Microscopic examination was performed on hematoxylin- and eosin-stained sections of brain tissue from rabbit pups of Project No. 466-103, removed by Caesarean section on Gestation Day 29 or 30. Ten of the pups (five male and five female, Path No. 47-738 to Path No. 47-747) were removed from does which had been fed monosodium glutamate at a level of 8.25% for two to three weeks prior to mating and during the subsequent 29 to 30 days prior to Caesarean section. The 10 control pups (five males and five females) were obtained from does which were maintained on the basal laboratory diet. Transverse sections of the brain were examined at the level of the hypothalamic nuclei and pituitary gland. Artifactual distortion of the brain was occasionally noted in sections from pups of both treated and control does. Evidence of neuronal necrosis or other pathologic alteration was not apparent in the sections examined.

In conclusion, there was no discernible difference in the histologic appearance of brain sections from pups obtained from control does or those receiving monosodium glutamate at a level of 8.25% of the diet.

Submitted by Richard W. Voelker, Jr.
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